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TOTAL ANTIOXIDANT CAPACITY AND ANTIHYPERLIPIDEMIC ACTIVITY OF ALKALOID EXTRACT FROM AERIAL PART OF *ANETHUM GRAVEOLENS* L. PLANT

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Abstract

Compounds of pharmacological interest (alkaloids) were extracted and the results revealed that the extract consist of three alkaloid compounds (A, B & C). Compound (A) has been isolated from *Anethum graveolens* L. aerial parts by column chromatography using C_6H_6 **:** Ethanol (9**:**1) solvent as eluent. It was found that simultaneous administration of alkaloid compound A (500 mg/kg/day) and cholesterol for 4 weeks, significantly ($P<0.01$) prevented the rise in serum TC, TG, LDL-cholesterol, VLDL-cholesterol, atherogenic index and significantly ($P<0.01$) increased TAC and HDL-cholesterol as compared to cholesterol fed control rabbits. Thus, the results indicate antioxidant and hypolipidemic effect of alkaloid compound A of *Anethum graveolens* L. aerial parts.

Keywords: Anethum graveolens L., Total Antioxidant Capacity, Antihyperlipidemic

Introduction

The alkaloids represent a group of natural products that had a major impact throughout history on the economic, medical, political and social affairs of humans. Many of these agents have potent physiological effects on mammalian systems as well as other organisms, and as a consequence, some constitute important therapeutic agents. Atropine, morphine, quinine and vincristine are representative of a host of agents used to treat a range of disease conditions that range from malaria to cancer. Therefore determination of total alkaloids is very important related to the quality of

medicinal plants (Shamsa et al., 2008). Lipids and lipoproteins abnormalities are preceding risk factor for cardiovascular diseases and prevalence of this in general population has increased considerably in last few decades. Hyperlipidemia contributes significantly in the prevalence and severity of atherosclerosis and coronary heart diseases (Gosain et al., 2010). Cardiovascular disease is a major cause of morbidity and mortality all over the world (Gupta and Jain, 2009). Increasing level of lipid leads to cardiovascular diseases and stroke. The underlying mechanism is increased LDL cholesterol, which further undergoes oxidative modification in the presence of free radicals (Agardh et al., 1999). The decrease LDL level with raised HDL cholesterol play important role against these complications. Due to the highly costs for synthetic drugs, probable side effects of this drugs and also restrictions in use of these drugs, during recent years scientists and researcher trying to substitute herbal plant and some natural component in these plants for treatment of disease (Hajizadeh et al., 2011). It is well established that consumption of fruit and vegetables prevents some diseases (Dauchet et al., 2006).

The present work aims to isolate the alkaloid compounds from *Anethum graveolens* L. and assess their possible total antioxidant capacity and antihyperlipidemic activity *in vivo*.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO), and solvents were from E. Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water (ddH2O) to eliminate the contamination of metal ions.

Plant material

The aerial parts of *Anethum graveolens* L. plant were collected during the month of September 2011 from the Abu-Al-Khaseeb region (southern Basra), Iraq. The plant was botanically authenticated and voucher specimens were deposited in the Herbarium of Basra (Iraq, Basra, College of Science, University of Basra). The plant was air-dried under shade at room temperature for 3 days, after which it was grinded to a uniform powder.

Preparation of extracts

Aqueous extract of *Anethum graveolens L.*

Anethum graveolens L. powder (100 g) was defatted with petroleum ether (40-60°C) at room temperature for 24 hours. Then, the defatted residue was refluxed with water for 24 hours. The residue was removed by filtration,

and the filtrate was concentration under vacuum, by freeze drier to afford 8.25g.

Alkaloids extract of *Anethum graveolens L.*

Fifty grams of the defatted ground of the *Anethum graveolens L.* aerial parts were extracted with methanol (100 mL) in a Soxhlet apparatus for 3 hours, and then evaporated to 0.5mL *in vacuo*. The methanolic residue was taken up in 10 mL of 2.5% hydrochloric acid and filtered. The aqueous acid solution was adjusted to pH=8 with concentrated ammonium hydroxide and extracted with dichloromethane (3 x 10 mL). The extracts were dried over magnesium sulphate and the solvent evaporated to afford 0.97g of crude alkaloid extract (Maiza-Benabdesselam et al., 2007).

Isolation of the components of alkaloids extract

Column chromatography was performed for separation of alkaloid component A (Spot with higher R_f = 0.86) from alkaloids extract. A glass column size (3×60 cm) was plug down to the bottom with small glass wool, then packed with HCl-washed silica-gel (mesh 230-400 μm). The slurry was prepared by dissolving 125g of silica-gel in 200ml of C_6H_6 **:** Ethanol (9**:**1) as eluent. The solid residue was then loaded to the top of the column and fractions of 5mL were collected and monitored by TLC. Fractions with the similar R_f were collected and dried at room temperature (Rispail et al., 2005).

phytochemical screening of *Anethum graveolens L.*

The different phytochemical tests were carried out using standard laboratory techniques. Alkaloids, carbohydrates, glycosides (Salkowski test) and saponins (Frothing test) were identified following the method of Sofowora, (1993). The presence terpenoids, steroids, flavonoids and tannins were tested using the method of Trease and Evans, (1989). Identification of carbohydrates and proteins was by the method of Khandelwal (2005).

Thin layer chromatography (TLC)

The foregoing prepared alkaloid extract and the alkaloid compound A of *Anethum graveolens L.* were tested for Thin layer chromatography (TLC). A known volume (on drops) of this extract (1mg/1mL) were spotted on TLC silica gel plate (2×10 cm) using C_6H_6 **:** Ethanol (9**:**1) as a solvent system. After 15 minutes, the plates were dried using hair drier and examined under long wavelength 366nm ultraviolet.. The plates were also stained with Dragendorff's, Mayer's and Wagner's reagents (Harborne, 1991).

Determination of toxicity (LD_{50}) of the alkaloid compound A

Forty mice were divided into five equal groups were used. Each group contains 8 mice (4male and 4 female). The first group one given 1ml of normal saline and was considered as a control group, while the second, third, fourth and fifth groups were given 2, 4, 6 and 8gm/kg body weight of alkaloid compound A respectively, dissolved in 1ml of normal saline. The last four groups were considered as treatment ones. Room temperature was controlled at 25±2°C with light for 12hrs, then the number of dead mice or any physical signs of toxicity such as writhing, gasping, palpitation, and decreased respiratory rate were recorded during period of 24, 48 and 72 hrs (Rattanakhot et al., 2007).

Experimental animals and design

Eighteen (18) adult, healthy, male albino rabbit, weighning 1.2-1.5 kg. were used in the study. All rabbits were housed at the animal house of College of Veterinary Medicine, Basrah University. The animals were housed individually in standard metallic wire gauge cages under standard condition (light period 12.00a.m. to 12.00p.m., 25±2°C, relative humidity 55% and water available *ad labium*. After acclimatization for 10 days they were divided into three groups of six animals each. Animals of Group I were fed on normal standard rabbit chow. Animals of Group II and III were made hypercholesterolemic by feeding them in addition to the normal diet, 2g/kg cholesterol powder mixed in 5ml soybean oil daily for 4 weeks (Gosain et al., 2010). Group III rabbits were also treated orally with (500 mg/kg/day) suspended in 1ml double distilled water simultaneously with cholesterol for 4 weeks.

Blood collection and serum biochemical analysis

At the end of the study (4 weeks), rabbits were starved for 12 h before anesthetizing with diethyl ether. The blood (about 1-2 ml) was collected directly from the heart by syringes, centrifuged at 4000 rpm for 10 minutes and the serum was obtained and stored at -20°C for the biochemical estimation. The plasma total cholesterol, triglyceride and HDL-cholesterol, were evaluated by enzymatic test kits (Randox Co). The LDL-cholesterol level was calculated by using the formula**:** LDLc= total cholesterol – [HDLc + (triglyceride/5)]**,** where (triglyceride/5) = VLDL-cholesterol (Fridewald et al., 1979). Total antioxidant capacity (TAC) was measured according to the method of (Koracevic et al., 2001). This method is used to measure the reductive power of a sample. It is based on suppression of the production of TBARS (Thiobarbituric acid reactive substances) in the presence of antioxidants. Briefly, the reactive mixture contained 0.5mL of a (10 mmol/L) Na-Benzoate, 0.2mL of H_2O_2 (10 mmol/L), 0.49 mL of phosphate buffer

(100 mmol/L, pH=7.4) (prepared by mixing 19.5 mL of KH_2PO_4 (100 mmol/L) with 80.5 mL of Na_2HPO_4 (100 mmol/L), then adjusted the pH to 7.4) and 0.2 mL of Fe-EDTA complex (2mmol/L) (prepared freshly by mixing equal volumes of EDTA (2mmol/L) and ammonium ferrous sulfate (2mmol/L), then left to stand at 25°C for 60 min)**.** Ten microliters of the blood serum was added to the latter reactive mixture and was incubated at 37°C for 60 min. Finally, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% w/v in 100 mL of 50mmole/L NaOH) was added and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100°C for 10 min. Total antioxidant capacity was calculated according to the following formula**:**

$$TAC\ (mmol/L) = (C_{UA})\ (K–A) / (K – UA)$$

Where**:**

(C_{UA})**:** Concentration of uric acid (mmol/L)**,** K**:** Absorbance of control ($K_1 – K_0$),

A**:** Absorbance of sample ($A_1 – A_0$), UA**:** Absorbance of uric acid solution ($UA_1 - UA_0$)**.**

Statistical analysis

The results are expressed as mean values ± SD and tested with analysis of variance followed by Student's *t*-test. P-values < 0.05, < 0.01 were considered to be statistically significant.

Results and Discussion

Qualitative analysis for all extracts of *Anethum graveolens L.* plant

The phytochemical screening of the aqueous extract of the earl part of *Anethum graveolens L.* showed the presence of the following**:** tannins, alkaloids, saponins, flavonoids, proteins and carbohydrates. The results however showed that glycosides, terpenoids and steroids were absent (**Table 1**). Same table indicate that the alkaloid extract contains only alkaloid compounds. Alkaloids usually have marked physiological action on human or animals. Saponins on the other hand are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D and cardiac glycosides. Tannins like alkaloids are substances which show protein precipitation and are related to the physiological effects of herbal medicines. Flavonoid containing plants have influence on arachidonic acid metabolism, thus could have anti-inflammatory, antiallergic, antithrombotic or vasoprotective effects (Evans, 2002). The implication of all these is that this plant is of great medicinal importance. The presence of alkaloides in this plant has further confirmed its medicinal use as antihyperlipidemic agent. The results presented in **Figure (1)** and **Table (2)** shows that the alkaloid extract of *A.*

graveolens L. contains three components. These components are related to the alkaloid family because they give a positive test only with Dragendorff's, Mayer's and Wagner's reagents (Bruno and Svoronos, 2003). The results revealed that the isolated alkaloid compound A by column chromatography technique, is one compound belong to the alkaloid family.

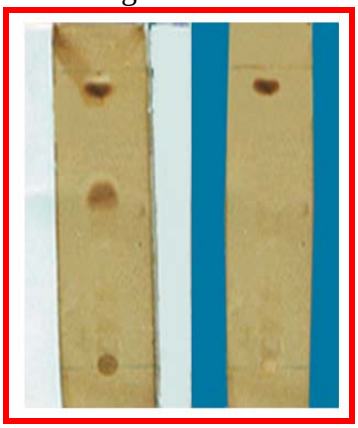

Figure 1: Thin layer chromatography for alkaloid extract and alkaloid compound A of *Anethum graveolens L.* plant

Table 1: Qualitative analysis for all extracts of *Anethum graveolens L.* plant

Reagents	Extracts			Results
	Aqueous	Alkaloids	Compound A	
Benedect	-	-	-	No Glycosides
Molisch	+	-	-	Carbohydrates are present
Iodine	-	-	-	No Polysaccharides
Dragendroff	+	+	+	Alkaloids are present
Mayer	+	+	+	
Wagner	+	+	+	
Libermann-Burchard	-	-	-	No Steroids
Frothing test	+	-	-	Saponins are present
Salkoviski	-	-	-	No terpenoids
1% $FeCl_3$	+	-	-	Tannins are present
Biuret	+	-	-	Proteins are present
10% lead acetate	+	-	-	Flavonoids are present
Mg ribbon	+	-	-	

Table 2: Thin layer chromatography for all extracts of *Anethum graveolens L.* plant

Reagents	UV 366 nm		Dragendorff's reagents		Mayer's reagents		Wagner's reagents	
Type of extract	**KE**	**KC A**	**KE**	**KC A**	**KE**	**KC A**	**KE**	**KC A**
No. of spots	0.86 0.34 0.01	0.86	0.86 0.34 0.01	0.86	0.86 0.34 0.01	0.86	0.86 0.34 0.01	0.86
Results	Conjugated system is present		Alkaloids is present		Alkaloids is present		Alkaloids is present	

Where, KE: alkaloid Extract and KC A: alkaloid compound A

The toxicity and effect on general behavior of alkaloid compound A

The mice treated with 2, 4, 6 and 8gm/kg body weight of alkaloid compound **A** did not show any drug induced physical signs of toxicity during the whole experimental period and no mortality was registered. Only behavioral changes observed included slightly weight loss and diarrhea. These early symptoms subsequently disappeared.

Total antioxidant capacity and antihyperlipidemic effect of alkaloid compounds (A) of
***A. graveolens* in normal and cholesterol fed rabbits**

The results of the present study demonstrated the total antioxidant capacity and hypolipidemic effect of alkaloid compounds (A) of *Anethum graveolens* in normal and cholesterol fed rabbits, **Table (3)**. During the induction of hyperlipidemia, the increase in body weight and amount of food intake were not significantly different among all groups (control, cholestrol-fed and treated group) of the study. Compared to the normal control group I, Serum TC, TG, LDL-cholesterol and VLDL-cholesterol levels increased significantly ($P<0.01$) in untreated control group II**,** whereas TAC and HDL-cholesterol has been significantly ($P<0.01$) reduced in this group. Furthermore, the total cholesterol/HDL-cholesterol ratio and LDL-cholesterol/HDL-cholesterol ratio were also increased significantly ($P<0.01$). Treated group III with alkaloid compound A (500 mg/kg/day) and cholesterol for 4 weeks, decreased TC, TG, LDL-cholesterol VLDL-cholesterol and increased TAC and HDL-cholesterol significantly as control feeding group II. The ratios total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol were also declined significantly ($P<0.01$) as compared to cholesterol fed control rabbits.

Table 2: Effect of alkaloid compound's (A) of *Anethum graveolens* on some serum parameters levels in normal and cholesterol fed rabbits

Groups	Dose mg/kg	Fasting serum parameters levels Values are mean ± SEM (n=6)							
		Total cholesterol (mg/dl)	TAC (mmol/l)	TG (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	LDLc/ HDLc ratio	TC/ HDL ratio
Group Ⅰ	Normal Control	122.18± 4.83	1.58 ±0.4	96.1±2 .34	55.09± 2.93	47.87± 2.71	19.22± 0.85	0.86± 0.34	2.21± 0.07
Group Ⅱ	Choles. fed Control	669.75± 18.7 [b]	0.83 ±0.3 [b]	285.4± 12.1 [b]	45.27± 7.8 [b]	567.4± 21.6 [b]	57.08± 6.4 [b]	12.53± 2.01 [b]	14.79± 2.1 [b]
Group Ⅲ	500	387.31± 24.7**	1.34 ±0.4 **	155.48 ±8.3**	78.2± 7.1**	278.02 ± 13.7**	31.09± 2.7**	3.55± 0.45**	4.95± 0.96**

c = cholesterol, TG = triglycerides, TAC = total antioxidant capacity and Choles. fed = cholesterol fed control. [a] $P<0.05$, [b] $P<0.01$ when compared with normal control rabbits and *$P<0.05$, ** $P<0.01$ when compared with cholesterol fed control rabbits.

Simultaneous Administration of alkaloid compound A of *Anethum graveolens* extract caused a significant decrease in serum total cholesterol, LDL-cholesterol, VLDL-cholesterol suggesting beneficial modulatory influence on cholesterol metabolism and turnover. Decline in the ratios of total cholesterol / HDL-cholesterol and LDL-cholesterol / HDL-cholesterol observed in the extract treated rabbits might be a consequence of higher proportion of HDL-cholesterol which reduced atherogenic risk by virtue of increased reverse cholesterol transport from peripheral organs to liver (Hermansen et al., 2003). Elevated serum triglycerides are considered as independent risk factor for cardiovascular disease (Dwivedi, 2004). A significant decline in the serum triglycerides level observed in plant extract treated rabbits supports the cardiovascular protective influence. Therefore, possible explanations for hypolipidemic effects of the alkaloid compound A of *Anethum graveolens* included that this compound could inhibit carbohydrate absorption and metabolism (Gosain et al., 2010). The cholesterol lowering effect of the plant extract is possibly associated with a decrease in intestinal absorption of cholesterol resulting in an increase in fecal excretion of neutral lipids (Purohit and Vyas, 2006). Also, it might be hypothesized that alkaloid compound A could inhibit lipogenesis, which in turn helps in treating type-IV and type-V hyperlipidemia. A large number of allopathic hypolipidemic drugs are currently available in the market but these lag behind the desired properties such as efficacy and safety on long term use, cost and simplicity of administration. These factors do not fulfill conditions for patients compliance (Davidson and Tooth, 2004). Plants and herbs are mines of large number of bioactive phytochemicals that might serve as lead for the development of effective, safe, cheap novel drugs. A number of medicinal plants have shown their beneficial effect on the cardiovascular disease (CVD) by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects (Dwivedi, 2004). A significant elevation in the level of lipid profile in cholesterol fed rabbits might be lead to increased the level of lipid peroxide (TBARs) and indicates enhanced oxidative stress in hyperlipidemic state which implicates in development and progression atherosclerotic lesions in aorta (Prasad, 2005). Administrations of alkaloid compound A decreased lipid peroxidation in these tissues indicating antioxidant like activity which alleviates oxidative stress. It was shown that reactive oxygen species (ROS) and other oxidants could be also formed in the normal physiological process (Onder and Gurer, 2001). Many antioxidant molecules found in blood prevent or inhibit the harmful effects of free radicals. Whenever there is a decrease in antioxidants and/or an

increase in oxidants, oxidant/antioxidant balance is impaired in favor of oxidants and this is known as oxidative stress (Isik and Koca, 2006). It is known that oxidative stress is responsible for tissue injury in many diseases and contributes to the development of atherosclerosis (Abuja and Albertini, 2001). Antioxidant activity indicates the antioxidant characteristic of only one antioxidant, whereas total antioxidant capacity (TAC) represent the total antioxidant characteristic of all antioxidants found in plasma. It is doubtlessly more advantageous to evaluate TAC, instead of individual antioxidant activities. Many methods have been developed recently for this aim. Total radical trapping antioxidant parameter (TRAP), oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) are colorimetric methods previously developed to assess TAC (Erel, 2004_a) (Erel, 2004_b) (Ghiselli et al., 2000). Total antioxidant status or total antioxidant capacity within a matrix such as food extracxt, beverage or body fluid (plasma, synovial fluid etc.) should reflect contribution to reducing properties of the individual antioxidants or electron-donating components. Thus, in some instances, total antioxidant potential might indeed be more informative than knowledge of the levels of individual antioxidant constituents per second (Rahbani–Nobar et al., 1999).

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